

**Paragraph 73**

The second round of hybridization used other allele-specific Luminex bead-probes as follows (SEQ ID NOS: 6-13):

Luminex bead-probes used to confirm allele specific separation

AS

L5'A107A	1AGGTATTTCTACACCTCCGTG
L5'A107C	1AGGTATTTCTCCACATCCGTG
L5'A153A	1CTTCATCGCAGTGGGCTAC
L5'A153C	1CTTCATCGCCGTGGGCTAC
L5'A249T	1GCAGGAGGGTCCGGAGTAT
L5'A249G	1GCAGGAGGGGCCGGAGTAT
L5'A291C	1GAAGGCCCACTCACAGACT
L5'A291G	1GAAGGCCCACTCACAGACT

✓  
Please insert the Sequence Listing filed concurrently herewith following the claims, renumber pages 1-5 of the Sequence Listing as pages 31-35 and renumber original page 31 as page 36.

**REMARKS**

Enclosed is an initial computer readable form of the Sequence Listing as required by the Notice to File Missing Parts Of Nonprovisional Application (Notice to File Missing Parts). Also enclosed is an initial paper copy of the Sequence Listing. Applicants submit this Amendment to insert the required references to SEQ ID NOS of the Sequence Listing filed concurrently herewith, to indicate the insertion point for the Sequence Listing and to effect the necessary changes in pagination. Applicants respectfully request examination on the merits of this application. A marked up copy of the changes to the specification is attached showing additions in bold and underline.


The time for response to the Office Action is set to expire as of November 20, 2001, and therefore this response is timely filed. A check in the amount of \$1,018.00 is enclosed to partially cover the fees required to comply with the Notice to File Missing Parts.

Additionally, please charge \$38.00 to Deposit Account No. 06-1447 to make up for the deficiency in the check. The Commissioner is hereby authorized to charge any additional fee or credit any overpayment in connection with this submission to Deposit Account No. 06-1447. A duplicate copy of this response is enclosed for these purposes.

Respectfully Submitted,

November 20, 2001

Date

  
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Version with markings to show changes made.

**Paragraph 10**

FIG. 1 is a diagram which illustrates allele identification utilizing an allele specific primer extension methodology according to the present invention. (SEQ ID NOS: 14-18 are shown in this figure.)

**Paragraph 53**

A 158 bp DNA fragment of HLA-A locus was amplified using sense primer 5' A200A and antisense primer 3' A322-1 with various genomic DNA samples obtained from UCLA registries (UCLA 210, UCLA 230 and UCLA 243). The 158 bp fragment was produced for this example using standard amplification methods. Primers used to amplify both Homo and Heterozygous DNAs in this example were (SEQ ID NOS: 1-2):

5'A200A	5' -ACA GCG ACG CCG CGA GCC A- 3' position 182 - 200, sense primer
3'A322-1	5' -CCTCGCTCTGGTTGTAGTA- 3' position 322 - 340, antisense primer

**Paragraph 57**

Using the single base extension reaction in an attempt to capture a specific allele; Allele Specific PCR was performed using Primer Mixes (PM), H001 and H002. These two primer mixes were used for the incorporation of specific bases at the site of the polymorphism. Both PM used a common 5' primer(agcgagcgccgagcca, SEQ ID NO:3), but used an allele specific 3' primer. PM H001 specifically incorporated the "C" (ccaagagcgaggtcctcg, SEQ ID NO: 4) base whereas PM H002 was specific for "A" (ccaagagcgaggtcctct, SEQ ID NO:5) at the respective sites of polymorphism, when a heterozygous DNA was used.

**Paragraph 68**

Different oligonucleotides for specific polymorphisms of the HLA A Locus were coupled to different bead sets (Luminex) to be used in the hybridization assay. The template that hybridized to the oligo coupled beads was selected to provide perfect sequence homology. Coupling beads to specific oligos was performed according to the manufacturer's

instructions (Luminex Corp.). The Luminex bead-probe conjugate were hybridized with PCR fragments produced above. The sequence of the probes used for separation of allele specific PCR fragments was (SEQ ID NOS:6-7):

L5'A107A	1AGGTATTTCT <u>A</u> CACCTCCGTG
L5'A107C	1AGGTATTTCT <u>C</u> CACATCCGTG

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L5'A153C	1CTTCATCGCCGTGGGCTAC
L5'A249T	1GCAGGAGGGTCCGGAGTAT
L5'A249G	1GCAGGAGGGGCCGGAGTAT
L5'A291C	1GAAGGCCCACTCACAGACT
L5'A291G	1GAAGGCCCA <u>G</u> TCACAGACT